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Does Aqueous Fullerene Inhibit the Growth of *Saccharomyces cerevisiae* or *Escherichia coli*?[▽]

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Studies reporting on potentially toxic interactions between aqueous fullerene nanoparticles (nC_{60}) and microorganisms have been contradictory. When known confounding factors were avoided, growth yields of *Saccharomyces cerevisiae* and *Escherichia coli* cultured in the presence and absence of independently prepared lots of underivatized nC_{60} were found not to be significantly different.

The increasing use of nanomaterials in industrial processes and commercial products is expected to lead to accumulation of these materials in the environment. Because the consequences of increased environmental exposure are unclear, it is important that studies be undertaken to determine potential risks (4). Both deleterious effects (5, 21, 26, 27, 30) and a lack of toxicity (7, 14, 18) have been reported for aqueous nanoparticles of underivatized aqueous fullerene nanoparticles (nC_{60}). Only some of these conflicting observations have been rationalized (9). Accurately assessing doses of nanoparticles in cell culture systems can be problematic (24). The variety of methods used to prepare nC_{60} also complicates interpretations of otherwise similar toxicological evaluations, because different preparation methods produce nC_{60} particles with different physicochemical properties (2, 16).

With specific reference to microorganisms, conflicting data have also been previously reported (19). At least four factors confound assessments of toxicity. First, it is now recognized that tetrahydrofuran (THF) used in nC_{60} preparation generates toxic derivatives (13, 22, 29). Unless these derivatives and trace THF are removed, observed toxicity cannot be ascribed to nC_{60} alone. Reports from studies that found antibacterial activity by the use of THF-solubilized nC_{60} prior to this discovery are thus difficult to interpret (6, 15–17). Second, in aqueous media, hydrophobic nC_{60} particles tend to agglomerate as a function of the solution condition. For some microbiological media, this leads to precipitation of nC_{60} (17) and hence a reduction in the actual exposure dose. Binding of organic components in complex media to nC_{60} particles can reduce nC_{60} bioavailability or lead to agglomeration (15). Both effects would result in false-negative assessments of potential growth inhibition (3). Third, negative results reported from studies where C_{60} powder was used directly without prior solubilization may reflect a lack of bioavailability (8, 20, 23, 25). Fourth, potential inhibitory effects toward one or few species in mixed cultures could be masked by other species when growth is assessed at the community level (8, 20, 25).

In light of these complications and a lack of studies done with fungi, which comprise a significant component of the soil microbial community, the toxicity of nC_{60} towards the yeast *Saccharomyces cerevisiae* and *Escherichia coli* was assessed based on a simple growth endpoint under conditions where the aforementioned confounding factors were avoided. Pure microbial cultures were grown in minimal media to which carefully washed and characterized independent lots of nC_{60} prepared using three different methods were added. At the single high dose used (about 30 μg/ml), no reduction in the cell yield of either *S. cerevisiae* or *E. coli* was observed. To our knowledge, this is the first report of a lack of microbial growth inhibition under conditions where factors known to generate false-negative results were avoided.

Preparation and characterization of aqueous fullerene suspensions. Aqueous nC_{60} suspensions were prepared with sublimed C_{60} powder (MER Corp., Tucson, AZ) (purity ≥ 99%) by three methods. The suspensions were termed tol-nC_{60}, THF-nC_{60}, and aq-nC_{60}, with the prefix indicating the solvent used in the preparation (toluene, THF, and water, respectively). Three parallel samples were prepared for each suspension type. A numerical suffix indicates the particular batch. C_{60} concentrations were determined by total organic carbon measurements using a high-sensitivity TOC analyzer (Shimadzu Scientific Instruments, Columbia, MD). All nC_{60} preparations were processed through a sterile filter with a 0.45-μm-pore-size membrane prior to use.

Tol-nC_{60}. Three 10-ml solutions of C_{60} in toluene at 2 g/liter were filtered through 0.45-μm-pore-size nylon filters (Millipore, Billerica, MA) and added to 100 ml of ultrapure water. Toluene was evaporated by continuous sonication at 100 W using a cell disruptor probe (Sonics and Materials, Inc., Newtown, CT), and the resulting aqueous suspensions were passed through 0.45-μm-pore-size sterile membrane filters and stored at 4°C in the dark. Residual toluene concentrations measured by gas chromatography and mass spectrometry (GC/MS) were less than 0.2 ppm.

THF-nC_{60}. THF-nC_{60} samples were prepared following a protocol that removes residual THF and toxic byproducts (29). Samples were washed repeatedly using ultrapure water in an Amicon stirred cell (Millipore, Billerica, MA) equipped with an ultrafiltration membrane (YM-10; Millipore, Billerica,

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MA). Residual THF concentrations measured by GC/MS were 1.8, 6.4, and 0.5 ppm in THF-nC_{60}1, THF-nC_{60}2, and THF-nC_{60}3, respectively.

Aq-nC_{60}. Aliquots of dry C_{60} powder (50 mg) were mixed with 200 ml of ultrapure water in autoclaved 500-ml glass bottles and vigorously stirred in the dark for 28 days. The resultant suspensions were filtered through 0.45-μm-pore-size sterile membrane filters and concentrated using centrifugal concentrators (Centricon YM-10; Millipore, Billerica, MA).

Particle sizes and electrophoretic mobilities (EPM) of nC_{60} particles were determined using a Zeta-sizer Nano (Zen 3600; Malvern Instruments, Worcestershire, United Kingdom) at 25°C. All samples were prepared in triplicate at 3 mg/liter in the corresponding background solutions used in the toxicity tests. Measurements were performed over a 24-h period immediately following sample preparation to monitor changes in particle size and EPM. At each time point, samples were measured at least five times for particle size and 10 times for EPM. The refractive index of nC_{60} was set at 2.20 for the particle size measurements (2).

Figure 1 summarizes the mean particle size and EPM measurements for all nC_{60} suspensions. All suspensions were very stable in deionized water, with insignificant changes in particle size over the period of the study (data not shown). In general, tol-nC_{60} particles were the smallest and aq-nC_{60} particles were the largest (Fig. 1A), which is consistent with previous findings (2). All suspensions were highly negatively charged in deionized water (Fig. 1B). The THF-nC_{60} samples had the highest negative EPM, while that of the aq-nC_{60} was the lowest, as in previous reports (2). There was little variation among the replicate preparations, except for aq-nC_{60}3, which exhibited notably lower negative EPM compared to the other two aq-nC_{60} samples. The direct mixing method used to prepare aq-nC_{60} was the least reproducible.

When mixed with growth media (defined below), the negative EPM of all samples was reduced due to the high salt concentrations (Fig. 1B). The reduction was greater in yeast nitrogen base without amino acids (Difco) that was supplemented with 2% glucose, 20 μg/ml histidine, 30 μg/ml each of leucine and lysine, and 10 μg/ml of uracil (henceforth referred to as YNB) than in a reduced-phosphate minimal medium (1; henceforth referred to as MD) because of the higher total ionic strength (142 mM in YNB versus 48 mM in MD) and divalent cation concentration in YNB. As a result, particle aggregation occurred, as indicated by the larger particle sizes after 24 h compared to those in deionized water. Particle aggregation was notably greater in YNB than in MD, consistent with the lower negative EPM in YNB. Aggregation of nC_{60} depended on the sample type. Aggregation in YNB was much greater for THF-nC_{60} than for the other two types, even though the EPM was similar to or more negative than those of tol-nC_{60} and aq-nC_{60}. This suggests that the reduction in electrostatic repulsion was not the only cause of particle aggregation; the surface chemistries of the various nC_{60} types may differ from one another. Despite the aggregation, there was no notable precipitation of nC_{60} over a 48-h period.

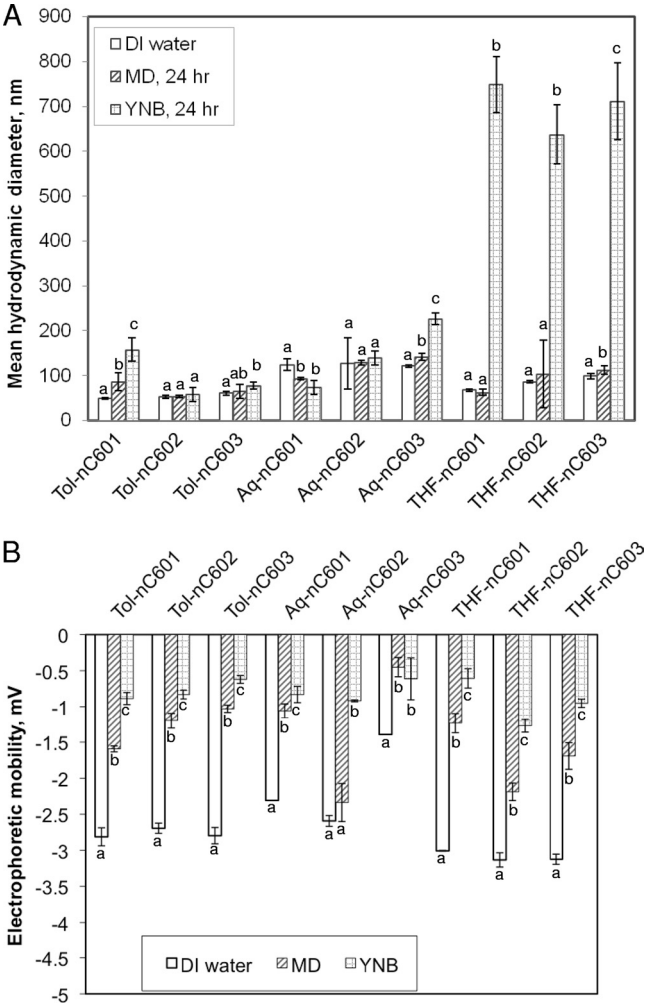

FIG. 1. Comparison of mean particle diameters and electrophoretic mobilities for all nC_{60} suspensions in deionized water and in the two growth media, YNB and MD, as described in the text. (A) Mean particle sizes measured after 24 h of mixing. (B) Electrophoretic mobility measured after 30 min of mixing in growth media. For each sample lot, data bars not sharing the same letter label are statistically different at $P < 0.05$ (Wilcoxon–Mann-Whitney 2-tailed test; XLSTAT 2009.1.02).

Microorganisms, media, and growth assays. *Saccharomyces cerevisiae* BY4742 (*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*) and a number of cell wall mutants in the BY4742 genetic background and *E. coli* DH5α were used to assess the growth-inhibitory activity of fullerene. The yeast mutants (Open Biosystems, Inc.) have been previously described (28) (http://sequence-www.stanford.edu/group/yeast_deletion_project/deletions3.html). *E. coli* DH5α was chosen specifically because it has been used in previous studies of nC_{60} toxicity (6, 17, 29). *S. cerevisiae* was chosen as a model microbial eukaryote and constituent of the soil microbial community. *S. cerevisiae* was grown in YNB. *E. coli* was grown in MD, with a 90% reduction in phosphate as described previously (18), consisting of 0.9 g of potassium phosphate, 1 g of ammonium sulfate, 0.5 g of sodium citrate dihydrate, 0.1 g of magnesium sulfate septahydrate, and 2 g of glucose per liter (pH 7). Liquid media were sterilized by filtration through 0.45-μm-pore-size membrane filters.

Yeast cells were subjected to aerobic preculturing for 24 h at 30°C at 200 rpm in 1 ml of YNB, centrifuged (12,000 × *g* for 20 s), washed twice in distilled water, resuspended in 1 ml of

TABLE 1. Cell yields of *E. coli* and *S. cerevisiae* grown in the presence of nC_{60}

nC_{60} lot	A_{600} value (means ± SD)[a]			
	E. coli DH5α		*S. cerevisiae* BY4742	
	Control	Treated (26 μg/ml)	Control	Treated (31 μg/ml)
Tol-nC_{60}1[b]	0.247 ± 0.027	0.238 ± 0.030	1.963 ± 0.111	1.940 ± 0.042
Tol-nC_{60}2	0.084 ± 0.013	0.095 ± 0.024	1.907 ± 0.016	2.068 ± 0.094
Tol-nC_{60}3	0.084 ± 0.013	0.085 ± 0.011	2.092 ± 0.022	2.187 ± 0.185
Aq-nC_{60}1	0.103 ± 0.008	0.108 ± 0.010	1.699 ± 0.019	1.683 ± 0.085
Aq-nC_{60}2	0.103 ± 0.008	0.106 ± 0.007	1.699 ± 0.019	1.888 ± 0.140
Aq-nC_{60}3	0.103 ± 0.008	0.093 ± 0.004	1.699 ± 0.019	1.833 ± 0.190
THF-nC_{60}1	0.107 ± 0.010	0.135 ± 0.004	1.656 ± 0.042	1.652 ± 0.044
THF-nC_{60}2	0.107 ± 0.010	0.138 ± 0.008	1.656 ± 0.042	1.725 ± 0.120
THF-nC_{60}3	0.107 ± 0.010	0.143 ± 0.005	1.656 ± 0.042	1.744 ± 0.071

[a] Data are means ± standard deviations for 3 replicates. Treated yeast and *E. coli* cells were grown in YNB and MD, respectively, containing nC_{60}. Control cells were grown in media lacking nC_{60}. No differences in yield between the control and treated cultures for any nC_{60} lot were significant at $P < 0.05$ (Wilcoxon–Mann-Whitney 2-tailed test; XLSTAT 2009.1.02).

[b] The growth yield of both control and treated *E. coli* cells was unexpectedly higher in the experiments performed to evaluate Tol-nC_{60}1 than in those performed with Tol-nC_{60}2 or Tol-nC_{60}3. Because the batch of medium used with control and Tol-nC_{60}1-treated cells was prepared independently from the single batch of medium used to evaluate both Tol-nC_{60}2 and Tol-nC_{60}3, we presume that batch differences in medium formulations may account for this difference in growth yield.

distilled water, and then diluted 1,000-fold in 250-μl aliquots of YNB (control) or YNB plus 31 μg/ml of nC_{60} fullerene in triplicate experiments to yield about 2×10^4 CFU/ml. Cells were incubated under conditions that were not strictly anaerobic in horizontal 1.5-ml screw-cap polypropylene tubes for 48 h at 30°C and 200 rpm. Growth was assessed as cell yield (A_{600}) by using the corresponding background nC_{60} suspension as the reference solution.

E. coli cells were subjected to aerobic preculturing for 24 h at 37°C at 200 rpm in 1 ml of MD, centrifuged (12,000 × *g* for 1 min), washed twice in 0.9% saline solution, resuspended in 1 ml of 0.9% saline solution, and diluted 1,000-fold in 1-ml aliquots of MD (control) or MD containing 26 μg/ml nC_{60} fullerene in triplicate experiments to yield about 2×10^4 CFU/ml. Cells were incubated under conditions that were not strictly anaerobic in horizontal 1.5-ml screw-cap polypropylene tubes for 24 h at 37°C and 200 rpm. Growth was assessed as cell yield (A_{600}) by using the corresponding background nC_{60} suspension as the reference solution.

Assessment of growth-inhibitory activity of nC_{60}. Inhibition of yeast or *E. coli* growth was assessed by comparing cell yields (A_{600}) in the presence and absence of nC_{60} (Table 1). No reduction in the cell yield of yeast or *E. coli* was observed for any of the 9 nC_{60} lots tested. While we are not aware of published data on the response of the widely used model eukaryote *S. cerevisiae* to fullerene, the *E. coli* results are inconsistent with two previous studies in which growth inhibition of the same *E. coli* strain in MD was observed at concentrations as low as 0.4 mg/liter of THF/nC_{60} (6, 17). However, as noted above and in references 13, 22, and 29, residual THF and toxic byproducts in the THF-nC_{60} preparation used in earlier studies (6, 17) cannot be ruled out as a cause of the reported toxicity. In contrast, the THF-nC_{60} used in the present study was washed as previously recommended (29). On the other hand, growth inhibition of *Bacillus subtilis* in MD has been reported from studies using nC_{60} preparations made without THF (16). Because our assay did not measure growth rates or changes in the viability of subpopulations of cells, it is possible that exposure to nC_{60} could have slowed growth of or irreversibly damaged some cells without affecting the maximum attainable population size. It was recently discovered that physical contact was required in order for single and multiwall carbon nanotubes to damage *E. coli* and other bacteria (10, 12). Forced physical contact between *E. coli* and an aq-nC_{60}-coated filter was reported to kill about 60% of nC_{60}-exposed cells (11). Unfortunately, making a rational comparison of cell-particle contact in the assay used in the present study to that in the forced contact assay is difficult.

Yeast cell wall mutants are not sensitive to nC_{60}. We speculated that growth inhibition would depend on fullerene uptake or association with cells and therefore assayed 48 yeast deletion mutants with known defects in cell wall biosynthesis or organization or with greater sensitivity or resistance to dyes that bind wall components (calcofluor white and Congo red) to determine whether they might be more susceptible. Growth of the deletion mutants was assayed as described above except that the 24-h inoculum was diluted 100-fold and only one lot of nC_{60} (31 μg/ml of tol-nC_{60}1) was tested. None of the observed modest differences in cell yield between the 48 strains grown in the absence versus the presence of tol-nC_{60} were significant at the $P < 0.05$ level (data not shown; Wilcoxon–Mann-Whitney 2-tailed test [XLSTAT 2009.1.02]). The 48 mutants tested are listed here according to the systematic names of the deleted genes: YAL059w, YBL001c, YBL006c, YBL007c, YBL043w, YBL061c, YBL101c, YBR005w, YBR023c, YBR067c, YBR076w, YBR078w, YCL005w, YDR125c, YDR245w, YDR446w, YER083c, YER093c, YGR189c, YGR229c, YHL043w, YHR021w, YHR030c, YHR132c, YHR142w, YHR181w, YIL146c, YJL201w, YJR075w, YJR106w, YJR137c, YKL096w, YKL190w, YKR076w, YLR110c, YLR300w, YLR332w, YLR342w, YLR390w, YLR425w, YLR436c, YLR443w, YMR238w, YMR307w, YOR008c, YOR092w, YPL089c, and YPL180w. We conclude that loss of these particular cell wall-related functions does not make *S. cerevisiae* more sensitive to nC_{60}-mediated growth inhibition.

To our knowledge, the present report is the first to present findings showing a lack of microbial growth inhibition by nC_{60} under conditions where nC_{60} remained in solution and solvent effects were avoided, factors that could have contributed to previous negative reports. In light of these findings and reports

of studies showing that damage requires physical contact between bacterial cells and carbon-based nanomaterials (10–12), the current suspension-based microbial toxicity assay needs to be carefully reexamined.

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REFERENCES

1. **Atlas, R. M.** 1993. Handbook of microbiological media. CRC Press, Boca Raton, FL.
2. **Brant, J. A., J. Labille, J. Y. Bottero, and M. R. Wiesner.** 2006. Characterizing the impact of preparation method on fullerene cluster structure and chemistry. Langmuir **22:**3878–3885.
3. **Chiron, J. P., J. Lamandé, F. Moussa, F. Trivin, and R. Céolin.** 2000. Effect of "micronized" C_{60} fullerene on the microbial growth in vitro. Ann. Pharm. Fr. **58:**170–175.
4. **Colvin, V. L.** 2003. The potential environmental impact of engineered nanomaterials. Nat. Biotech. **21:**1166–1170.
5. **Dhawan, A., J. S. Taurozzi, A. K. Pandey, W. Shan, S. M. Miller, S. A. Hashsham, and V. V. Tarabara.** 2006. Stable colloidal dispersions of C60 fullerenes in water: evidence for genotoxicity. Environ. Sci. Technol. **40:** 7394–7401.
6. **Fortner, J. D., D. Y. Lyon, C. M. Sayes, A. M. Boyd, J. C. Falkner, E. M. Hotze, L. B. Alemany, Y. J. Tao, W. Guo, K. D. Ausman, V. L. Colvin, and J. B. Hughes.** 2005. C_{60} in water: nanocrystal formation and microbial response. Environ. Sci. Technol. **39:**4307–4316.
7. **Gharbi, N., M. Pressac, M. Hadchouel, H. Szwarc, S. R. Wilson, and F. Moussa.** 2005. [60]Fullerene is a powerful antioxidant in vivo with no acute or subacute toxicity. Nano Lett. **5:**2578–2585.
8. **Johansen, A., A. L. Pedersen, K. L. Jensen, U. Karlson, B. M. Hansen, J. J. Scott-Fordsmand, and A. Winding.** 2008. Effects of C_{60} fullerene nanoparticles on soil bacteria and protozoans. Environ. Tox. Chem. **27:**1895–1903.
9. **Johnston, H. J., G. R. Hutchison, F. M. Christensen, K. Aschberger, and V. Stone.** 2009. The biological mechanisms and physicochemical characteristics responsible for driving fullerene toxicity. Toxicol. Sci. **114:**162–182.
10. **Kang, S., M. Herzberg, D. F. Rodrigues, and M. Elimelech.** 2008. Antibacterial effects of carbon nanotubes: size does matter! Langmuir **24:**6409–6413.
11. **Kang, S., M. S. Mauter, and M. Elimelech.** 2009. Microbial cytotoxicity of carbon-based nanomaterials: implications for river water and wastewater effluent. Environ. Sci. Technol. **43:**2648–2653.
12. **Kang, S., M. Pinault, L. D. Pfefferle, and M. Elimelech.** 2007. Single-walled carbon nanotubes exhibit strong antimicrobial activity. Langmuir **23:**8670–8673.
13. **Kovochich, M., B. Espinasse, M. Auffan, E. M. Hotze, L. Wessel, T. Xia, A. E. Nel, and M. R. Wiesner.** 2009. Comparative toxicity of C_{60} aggregates toward mammalian cells: role of tetrahydrofuran (THF). Environ. Sci. Technol. **43:**6378–6384.
14. **Levi, N., R. R. Hantgan, M. O. Lively, D. L. Carroll, and G. L. Prasad.** 2006. C60-fullerenes: detection of intracellular photoluminescence and lack of cytotoxic effects. J. Nanobiotech. **4:**14.
15. **Li, D., D. Y. Lyon, Q. Li, and P. J. J. Alvarez.** 2008. Effect of soil sorption and aquatic natural organic matter on the antibacterial activity of a fullerene water suspension. Environ. Toxicol. Chem. **27:**1888–1894.
16. **Lyon, D. Y., L. K. Adams, J. C. Falkner, and P. J. J. Alvarez.** 2006. Antibacterial activity of fullerene water suspensions: effects of preparation method and particle size. Environ. Toxicol. Chem. **40:**4360–4366.
17. **Lyon, D. Y., J. D. Fortner, C. M. Sayes, V. L. Colvin, and J. B. Hughes.** 2005. Bacterial cell association and antimicrobial activity of a C_{60} water suspension. Environ. Toxicol. Chem. **24:**2757–2762.
18. **Mori, T., H. Takada, S. Ito, K. Matsubayashi, N. Miwa, and T. Sawaguchi.** 2006. Preclinical studies on safety of fullerene upon acute oral administration and evaluation for no mutagenesis. Toxicology **225:**48–54.
19. **Neal, A. L.** 2008. What can be inferred from bacterium-nanoparticle interactions about the potential consequences of environmental exposure to nanoparticles? Ecotoxicology **17:**362–371.
20. **Nyberg, L., R. F. Turco, and L. Nies.** 2008. Assessing the impact of nanomaterials on anaerobic microbial communities. Environ. Sci. Technol. **42:** 1938–1943.
21. **Oberdörster, E.** 2004. Manufactured nanomaterials (fullerenes, C_{60}) induce oxidative stress in the brain of juvenile largemouth bass. Environ. Health Perspec. **112:**1058–1062.
22. **Spohn, P., C. Hirsch, F. Hasler, A. Bruinink, H. F. Krug, and P. Wick.** 2009. C_{60} fullerene: a powerful antioxidant or a damaging agent? The importance of an in-depth material characterization prior to toxicity assays. Environ. Pollut. **157:**1134–1139.
23. **Tang, Y. J., J. M. Ashcroft, D. Chen, G. Min, C.-H. Kim, B. Murkhejee, C. Larabell, J. D. Keasling, and F. F. Chen.** 2007. Charge-associated effects of fullerene derivatives on microbial structural integrity and central metabolism. Nano Lett. **7:**754–760.
24. **Teeguarden, J. G., P. M. Hinderliter, G. Orr, B. D. Thrall, and J. G. Pounds.** 2007. Particokinetics *in vitro*: dosimetry considerations for *in vitro* nanoparticle toxicity assessments. Toxicol. Sci. **95:**300–312.
25. **Tong, Z., M. Bischoff, L. Nies, B. Applegate, and R. F. Turco.** 2007. Impact of fullerene (C_{60}) on a soil microbial community. Environ. Sci. Technol. **41:**2985–2991.
26. **Usenko, C. Y., S. L. Harper, and R. L. Tanguay.** 2007. In vivo evaluation of carbon fullerene toxicity using embryonic zebrafish. Carbon N. Y. **45:**1891–1898.
27. **Usenko, C. Y., S. L. Harper, and R. L. Tanguay.** 2008. Fullerene C_{60} exposure elicits an oxidative stress response in embryonic zebrafish. Toxicol. Appl. Pharmacol. **229:**44–55.
28. **Winzeler, E. A., D. D. Shoemaker, A. Astromoff, H. Liang, K. Anderson, et al.** 1999. Functional characterization of the *S. cerevisiae* genome by gene deletion and parallel analysis. Science **285:**901–906.
29. **Zhang, B., M. Cho, J. D. Fortner, J. Lee, C.-H. Huang, J. B. Hughes, and J.-H. Kim.** 2009. Delineating oxidative processes of aqueous C_{60} preparations: role of THF peroxide. Environ. Sci. Technol. **43:**108–113.
30. **Zhu, S., E. Oberdörster, and M. L. Haasch.** 2006. Toxicity of an engineered nanoparticle (fullerene, C_{60}) in two aquatic species, Daphnia and fathead minnow. Mar. Environ. Res. **62:**S5–S9.